In Vivo Bleeding Time and In Vitro Thrombelastography Measurements are Better Indicators of Dilutional Hypothermic Coagulopathy Than Prothrombin Time

Bijan S. Kheirabadi, PhD, Jacqueline M. Crissey, BS, Rodolfo Deguzman, BS, and John B. Holcomb, MD

Background: **The coagulopathy of trauma is generally confirmed by prothrombin time (PT) ≥16 seconds or an international normalized ratio ≥1.5. However, the utility of these values as a screening test is unknown. We examined different coagulation tests to determine the best predictor of coagulopathic bleeding and mortality in a small animal hemorrhage model.**

Methods: **Coagulopathy was induced in male New Zealand White rabbits by warfarin (W; 2 mg/kg for 2 days; n = 7), or hemodilution and hypothermia (HH; 50% blood exchange with Hextend, 34.5 ± 0.3°C; n = 7). Normal (N) rabbits without pretreatment served as the control (n = 7). Blood samples collected after coagulopathy induction and analyzed by prothrombin time (PT), activated partial thromboplastin time (aPTT), and thromboelastography (TEG) tests. Liver bleeding time (BT) was also measured before injury. An uncontrolled hemorrhage was created by a longitudinal splenic incision and the abdomen was closed. Rabbits were resuscitated with Hextend solution (25 mL/kg) to return blood pressure to baseline and monitored for 2 hours or until death at which time blood loss was measured.**

Results: **Warfarin-induced coagulopathy increased BT, PT, and aPTT. TEG showed increased reaction (R) and clot formation (K) times and marked decrease in clotting rate (α angle and Vmax). Hemodilution hypothermia coagulopathy increased only BT and aPTT, and decreased the clotting rate (α angle and Vmax) and strength of the clot. After injury, blood losses were higher in coagulopathic rabbits (W = 54.6 ± 4.2 and HH = 51.1 ± 8.9 mL/kg) than in normal rabbits (30.6 ± 12.4 mL/kg) and resulted in 86%, 100%, and 0% death, respectively. BT and Vmax consistently predicted coagulopathic bleeding and death in all animals.**

Conclusion: **Although satisfactory in warfarin-induced coagulopathy, PT was not a valid screening test for dilutional and hypothermic coagulopathy. BT and TEG measurements of blood clotting rate are better indicators of coagulopathic bleeding and mortality in this lethal hemorrhage model.**

Key Words: **Coagulopathy, Warfarin, Hemodilution, Hypothermia, Hemorrhage model.**

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Hemorrhage is the second leading cause of death in trauma patients.[1,2] The inability to achieve hemostasis is either caused by a major cardiovascular injury or results from the development of coagulopathy. Coagulopathy, defined as the inability of blood to clot normally, occurs as a result of massive tissue injury or closed head injury, subsequent activation of tissue factor, and consumption of coagulation proteins. It is often worsened by massive blood or other fluid transfusion, metabolic acidosis, and hypothermia, or any combination of these factors. Coagulopathy has a significant impact on morbidity and mortality. Elevated prothrombin time (PT) and activated partial thromboplastin time (aPTT) are recognized as the main clinical indicators of coagulopathy. According to the British National Blood Transfusion Service and the American College of Pathologists, PT above 18 seconds, aPTT above 60 seconds, or thrombin time (TT) above 15 seconds indicate coagulopathy requiring blood product replacement therapy.[3,4] However, the point at which increases in PT, aPTT, or TT are considered indicative of coagulopathy differs among clinical studies. Cosgriff et al. defined life-threatening coagulopathy as PT and aPTT more than two times that of normal laboratory controls.[5] Brohi and his colleagues defined coagulopathy as PT, aPTT, or TT more than 1.5 times normal and, with Injury Severity Score (ISS), a significant predictor of mortality.[6] MacLeod et al. indicated that PT >14 seconds or PTT >34 seconds constituted coagulopathy.[7] Additionally, Aoki et al. identified PTT ≥80 seconds combined with severe acidosis (pH ≤7.2) at admission to the intensive care unit (ICU) as important predictors of mortality in patients who are severely injured undergoing damage control surgery.[8]

Hypothermia, a significant contributor to the development of coagulopathy, is known to be an independent risk factor for acute mortality and to increase resuscitation fluid requirements in trauma patients.[9] As many as 66% of seri-

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From the US Army Institute of Surgical Research, Fort Sam Houston, Texas.

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Address for reprints: Bijan S. Kheirabadi, PhD, US Army Institute of Surgical Research, 3400 Rawley E. Chambers Avenue, Fort Sam Houston, TX 78234-6315; email: bijan.kheirabadi@cen.amedd.army.mil.

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ously injured trauma patients arrive in emergency departments with hypothermia (36°C).[10] The effect of hypothermia, independent of the development of acidosis, has also been associated with prolonged clotting times and other coagulopathies after traumatic injury.[11–13] The hypothermic effect on coagulation is attributed to inhibition of enzymatic activities, manifested by prolonged PT, PTT, and platelet function. Prolonged PT has been reported in hypothermic patients and in experimental animals exposed to cold temperatures when their plasma samples were measured at the corresponding hypothermic temperatures.[14–16]

Another form of coagulopathy develops secondary to vitamin K deficiency.[17] ICU patients who receive intravenous fluids and remain on broad-spectrum antibiotics for extended periods without any food can develop vitamin K deficiency. This results in bleeding tendencies in the form of increased bruising, hematomas, and oozing from the recent surgical sites, or in massive hemorrhage from silent, pre-existing lesions such as peptic ulcers. Severe reduction of the vitamin K-dependent coagulation factors II, VII, IX, and X prolongs both PT and aPTT tests. Treatment with sodium warfarin has a similar effect on hemostasis. It inhibits the synthesis of vitamin K-dependent clotting factors and anticoagulant proteins C and S, resulting in an in vivo sequential depression of factors VII, IX, X, and II activity based on their half lives.

The purpose of this study was to develop an uncontrolled hemorrhage model in small animals and explore the outcome of the hemorrhage under normal and coagulopathic conditions. In addition, an objective was to perform different coagulation tests and determine the best predictor of coagulopathic bleeding and mortality in the model. Coagulopathy was produced by two different methods: warfarin treatment that mimicked vitamin K deficiency or by dilutional hypothermia procedure.

MATERIALS AND METHODS

The Animal Care and Use Committee of the U.S. Army Institute of Surgical Research approved this study. Male New Zealand White (NZW) rabbits, specific-pathogen-free, weighing 2.9 to 3.4 kg were used for this study. All animals received care in strict compliance with the *Guide for the Care and Use of Laboratory Animals*.[18] Before surgery, rabbits were acclimated for a minimum of 72 hours and carefully checked for preexisting diseases. After acclimation, blood samples were collected from the central ear artery and complete blood count (CBC) and standard coagulation parameters (PT, aPTT, fibrinogen) were measured to verify the health and normal hemostatic function of the animals. Automated clinical laboratory equipment was used for all the hematologic measurements. The ABX Pentra 120 CBC Analyzer (ABX Diagnostics, Irvine, CA) was used for CBC measurements of whole blood and the Dade-Behring BCS Coagulation Analyzer (Deerfield, IL) was used for measuring coagulation parameters and clotting factor activity in citrated plasma samples. Coagulation factor activity was determined by coagulometric methods using human factor II, VII, IX, and X deficient plasma for assays.

Twenty-one specific pathogen-free rabbits were randomly divided into three groups: normal (no pretreatment, N), warfarin-treated (W), and hemodiluted hypothermia (HH) groups (n = 7/group). The warfarin rabbits were pretreated with sodium warfarin (2 mg/kg/d) to induce a profound vitamin K-deficient coagulopathy. The animals were injected intravenously with sodium warfarin solution (0.4–0.5 mL; 15 mg/mL) for 2 to 3 days and their PT was checked regularly. Once laboratory tests determined PT as sufficiently prolonged (~2 times baseline), animals were enrolled into the hemorrhage experiments. In the hemodiluted hypothermic group, the preconditioning to induce coagulopathy was performed on the day of surgery before the hemorrhage experiment.

Animal Preparation and Instrumentation

The daily food rations were withdrawn from the surgical candidates on the day of surgery while maintaining free access to water. Anesthesia was induced with intramuscular (IM) injections of fentanyl citrate (0.05 mg/kg) and midazolam (~2 mg/kg) into the hind legs. The marginal veins in both ears were cannulated with 24 G, 1.5-inch intravenous (IV) catheters for fluid administration and anesthesia injection. Surgical anesthesia was produced and maintained by additional injections of a mixture of ketamine (15–25 mg/kg) and midazolam (1.5–2 mg/kg). This anesthetic regimen was further supplemented with IV injections of a short-acting barbiturate, 2% methohexital (0.2 mL; 20 mg/kg), as required during the laparatomy procedure. The rabbits received maintenance fluid of lactated Ringer's solution at 10 mL/kg/h before the splenic injury. Animal body temperature was monitored with a rectal thermocouple and maintained between 37°C and 38°C with the use of a heating pad. To prevent hypoxia, oxygen was provided at a rate of 1 L/min via a loose-fitting facemask (PaO_2 >100 mm Hg).

The right common carotid artery was cannulated with 18 G, 2 inch IV catheter and closed with an injection cap. An arterial blood sample was collected from the catheter and blood gas measurements, CBC, standard coagulation tests, and thromboelastogram analysis were performed at 37°C. A small gel-filled catheter attached to a precalibrated transducer (TL 11M2-D70-PCT, Data Sciences International St. Paul, MN) was then inserted into the injection cap and secured. Blood pressure (systolic, diastolic, and mean) and heart rate measured by the transducer were transmitted continuously to a receiver plate (wirelessly), and were displayed and recorded by a computer system for future analysis.

For the hemodilution and hypothermia procedure, an additional catheter (21 G; 2-inch length) was placed in the femoral artery and closed with a three-way stopcock. Next, 50% of the circulating blood volume was estimated based on the assumption that the rabbit blood volume was equal to 7% of its body weight[19] and the specific gravity of blood was equal to 1. Using a dual syringe pump (Harvard Apparatus,

Holliston, MA), the estimated 50% blood volume (105–125 mL) was withdrawn from the femoral artery at 5 mL/min while simultaneously infusing an equal volume of Hextend solution (25°C) by IV at the same rate. This procedure had no impact on the mean arterial pressure (MAP), heart rate, or breathing frequency of the rabbits. During the blood exchange, the rabbit's core temperature was allowed to drop 4°C below its normal level (38°C), after which it was maintained at approximately 34 ± 0.5°C. After a stabilization period of at least 10 minutes at this temperature, an arterial blood sample was collected and blood gas measurements, CBC, standard coagulation tests, and thromboelastography (TEG) analysis were performed. The clotting times were measured at 37°C and 34°C whereas the TEG assay was only run at 34°C for this posthemodilution sample.

Surgical Procedure

Laparatomy was performed via midline incision. Before the splenic injury, bleeding times were measured by piercing the middle and left liver lobes two to three times with a 5-mm blade microknife. The oozing blood was absorbed periodically by cotton tip applicators away from the wound until the bleeding stopped. The bleeding times from the injury sites were recorded and averaged for each measurement. Blood loss from these injuries was negligible (<1 mL). A 10-minute stabilization period was allowed and baseline blood pressure data recorded. A stable mean arterial pressure (MAP) of 60 mm Hg or higher was required before proceeding with the next phase of the experiment. To create the injury, a 5-mm deep incision was made along the length of the spleen's dorsal capsule with a microknife, taking care not to completely divide the spleen into two segments. After the injury, the peritoneal cavity was rapidly sutured closed to avoid any blood spillage. Ten minutes after the injury and hemorrhage, at which time blood pressure was substantially decreased, IV fluid resuscitation (Hextend) was initiated (1 mL/min) and targeted to return MAP to the baseline level. Fluid resuscitation was intermittent; at the target MAP (baseline) resuscitation was discontinued until pressure fell below 10% of the baseline level, at which time it was restarted. To avoid excessive hemodilution, however, only up to 25 mL/kg of the fluid was administered to each rabbit during the 2-hour observation period. At the conclusion of the experiment (120 minutes postinjury or at death), surviving animals were killed and the peritoneal cavity was reopened; blood and blood clots were collected with dry gauze and weighed to estimate the total blood loss.

Statistical Analysis

The Tukey-Kramer and analysis of variance statistical tests were used to compare the groups for their presurgical (screening) criteria. One-way analysis of variance (ANOVA) and nonparametric ANOVA (Kruskal-Wallis test) were used for comparison of hematologic, coagulation, and blood loss measurements among the groups. Dunnett's multiple comparison test was used as the post-test to compare pairs of group means. The comparison of survival times was performed using the Log rank test. The incidence of survival was compared using Fisher's exact test. Multiple logistic regression analysis with stepwise selection was performed on the coagulation measurements for determining the more sensitive predictors of lethal bleeding. Data in the tables are expressed as mean ± SD. Statistical significance was assigned at greater than 95% confidence level ($p < 0.05$).

Table 1 Rabbits Weight and Baseline Blood Measurements Prior to Inducing Coagulopathy

Variable	Mean ± STD
Body Weight (kg)	3.2 ± 0.1
White blood cells ($10^6/\mu L$)	8.3 ± 2.0
Red blood cells ($1000/\mu L$)	5.8 ± 0.5
Hemoglobin (mg/dL)	12.3 ± 0.9
Platelet ($1000/\mu L$)	298.9 ± 98.0
PT (second)	11.0 ± 0.6
aPTT (second)	16.7 ± 0.9
Fibrinogen (mg/dL)	176.6 ± 45.7

No significant difference was found among the rabbit groups (n = 21).

RESULTS

Before inducing coagulopathy, blood samples were collected from the rabbits and baseline CBC and standard coagulation parameters were determined. The average values for these measurements are shown in Table 1. No significant difference in any of the parameters was detected among the three experimental groups; therefore groups were combined to show baseline values.

Coagulopathy Induction

The warfarin treatment produced a profound coagulopathy, resulting in a 1.9-fold increase in PT and a 1.3-fold increase in aPTT of the plasma samples compared with of normal rabbits (Table 2). The red blood cell (RBC), platelet count, and fibrinogen concentration of the warfarin rabbits remained unchanged. These parameters, as well as the percent activities of the vitamin-K dependent coagulation factors (relative to standard human plasma), are shown in Table 2. The activity of these factors, particularly factor VII with the shortest half life (4–6 hours), was significantly diminished in warfarin-treated animals compared with in normal rabbits. The TEG analysis of the whole blood (Fig. 1) showed a significant increase in reaction time (R-time) and marked reduction in clot formation rate as measured by the decrease in α angle and maximum clotting velocity (Vmax) and increase in clotting time (K-time) and time to reach Vmax (t-Vmax). The maximum strength of clot (MA) was unchanged. The Vmax and t-Vmax were calculated from the first derivative of the upper fork of the TEG graphs.[20] The TEG data are summarized in Table 3.

Table 2 **Complete Blood Count, Coagulation, and Other Baseline Measurements of the Rabbits Prior to Splenic Injury and Hemorrhage (n = 7)**

Variables	N	W	HH	*p* Value
RBC (1,000/μL)	5.7 ± 0.6	5.9 ± 0.3	2.8 ± 0.2*	<0.01, HH vs. N or W
Hgb (mg/dL)	12.6 ± 1.1	12.3 ± 0.7	5.9 ± 0.8*	<0.05, HH vs. N; <0.01, HH vs. W
Platelet (1,000/μL)	298.7 ± 91.7	319.0 ± 124.1	150.6 ± 42.6*	<0.05, HH vs. N or W
PT† (second)	11.2 ± 0.7	20.9 ± 2.8*	11.8 ± 1.4	<0.01, W vs. N; <0.05, W vs. HH
aPTT^{+} (second)	16.6 ± 0.9	22.2 ± 3.4*	27.0 ± 10.7*	<0.01, HH vs. N; <0.05, W vs. N
Fibrinogen (mg/dL)	155.6 ± 29.7	199.2 ± 25.6	90.4 ± 23.7*	<0.01, HH vs. W
Factor II (% DN)	243.4 ± 53.7	103.5 ± 33.0*	106.5 ± 14.5*	<0.05, W or HH vs. N
Factor VII (% DN)	83.6 ± 21.1	22.5 ± 8.5*	45.7 ± 12.2	<0.01, W vs. N
Factor IX (% DN)	282.3 ± 48.2	121.7 ± 46.8*	126.2 ± 15.6*	<0.05, W or HH vs. N
Factor X (% DN)	142.6 ± 50.3	45.4 ± 14.7*	51.0 ± 12.7*	< 0.01, W vs. N; <0.05, HH vs. N
pH	7.3 ± 0.05	7.25 ± 0.07	7.33 ± 0.03	NS
Preinjury MAP (mm Hg)	82.9 ± 9.4	83.1 ± 9.9	78.9 ± 9.7	NS
Body Temp (°C)	37.6 ± 0.3	37.6 ± 0.3	34.5 ± 0.3*	<0.01, HH vs. N or W

* Statistically different than other group(s).

† The PT and aPTT of all plasma samples were measured at the standard 37°C. The PT and aPTT for HH samples were also measured at the hypothermic temperature (34°C) and were equal to 12.5 ± 0.4 and 25.1 ± 3.5 s respectively.

% DN, percent activity relative to Dade Normal human plasma activity; NS, not significant.

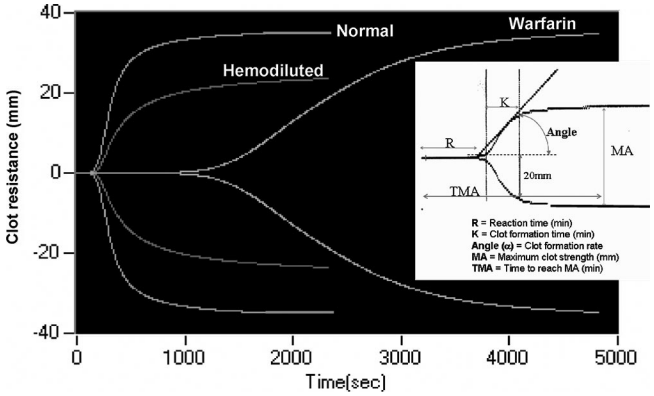

Fig. 1. *(A) Averages of the TEG tracings of normal and coagulopathic rabbits. (B) Schematic illustration of a TEG tracing.*

Trauma-related coagulopathy was produced based on significant hemorrhage and subsequent fluid replacement (hemodilution) and hypothermia without massive tissue injury or metabolic acidosis. A mild acidosis (respiratory, pH = 7.3) that was measured in this and other groups was likely a result of the elevated $PaCO_2$ levels (56.5 ± 4.1 mm Hg) that occurs in spontaneous breathing of anesthetized rabbits.[21] The arterial PaO_2 was greater than 100 mm Hg in all the subjects. The CBC and coagulation parameters after induction of coagulopathy compared with those changes in normal animals are shown in Table 2. Note that the 50% hemodilution with Hextend solution increased the aPTT (1.6 fold) but did not change the PT of the plasma samples. The activities of the clotting factors II, IX, and X were significantly diminished in hemodiluted samples compared with in the normal samples. The decrease in factor VII was not statistically significant. The TEG analysis of whole blood in this group (Fig. 1) showed significant decreases in clot formation rate (α angle)

Table 3 **TEG Results of the Rabbit Blood Samples Prior to Splenic Injury and Hemorrhage (n = 7/group)**

TEG Parameters	N	W	HH	*p* Value
Reaction time (min)	3.1 ± 0.7	25.9 ± 9.2*	3.4 ± 0.5	<0.01, W vs. N <0.05, W vs. HH
Clotting time (min)	1.2 ± 0.3	9.0 ± 3.8*	2.8 ± 1.0	<0.001, W vs. N
Clotting rate	74.1 ± 3.1	30.3 ± 9.3*	56.1 ± 6.1*	<0.001, W vs. N <0.05, W vs. N
Maximum strength (mm)	68.2 ± 4.3	69.3 ± 5.8	45.1 ± 4.5*	<0.01, HH vs. W or N
Maximum clotting rate (mm/min)	20.3 ± 4.5	3.9 ± 1.4*	8.2 ± 1.8*	<0.001, W vs. N <0.05, HH vs. N
Time to reach Vmax(min)	4.4 ± 0.9	39.4 ± 14*	4.4 ± 0.5	<0.01, W vs. N or HH

* Statistically different than other group(s).

N, normal rabbits; W, warfarin rabbits; HH, hemodilution and hypothermia rabbits.

Table 4 In vivo Bleeding Times and the Outcome of Splenic Bleeding in Rabbits (n = 7/group)

Measurements	N	W	HH	*p* Value
Bleeding time (seconds)	136.0 ± 23.7	310.3 ± 54.8*	266.2 ± 64.3*	<0.01, W vs. N <0.05, HH vs. N
Blood Loss (ml/kg)	30.6 ± 12.4	51.1 ± 8.9*	54.6 ± 4.2*	<0.05, W vs. N <0.01, HH vs. N
Survival Time (min)	120*	91.0 ± 23.0	66.7 ± 14.3	<0.001, N vs. W and HH
% Survival	100 (7/7)*	14 (1/7)	0 (0/7)	<0.001, N vs. W or HH

* Statistically different than other group(s).
N, normal rabbits; W, warfarin rabbits; HH, hemodilution and hypothermia rabbits.

and Vmax but no change in R-time or t-Vmax. The MA was also diminished in hemodiluted blood samples compared with in the normal controls (Table 3).

Bleeding Outcome

The in vivo bleeding time, measured shortly before splenic injury, was significantly prolonged in both coagulopathic groups (Table 4). The changes were more pronounced in warfarin treated rabbits (2.5 fold) than in the hemodiluted hypothermic rabbits (2 fold) compared with in the normal controls. The splenic injury resulted in widely different responses in blood pressure (Fig. 2) and bleeding severity between the normal and coagulopathic rabbits. Whereas there was no difference in the MAP of rabbits before hemorrhage, 10 minutes after the injury (before resuscitation was started), the MAP dropped to 58.0 ± 15.4 mm Hg in normal rabbits, to 44.3 ± 14.1 in W, and to 29.3 ± 5.0 mm Hg in HH coagulopathic rabbits ($p < 0.05$ vs. normal). The MAP of normal animals subsequently increased in response to fluid resuscitation, whereas little or no change in the pressure occurred in the coagulopathic groups. Fluid resuscitation, however, did not restore the baseline MAP, even in normal rabbits. Therefore, the rabbits from all three groups received the same amount of fluid resuscitation (25 mL/kg Hextend) unless the animal died at an early stage of the experiment.

The splenic injury in coagulopathic animals led to severe hemorrhage and exsanguination of all the HH and six of seven W rabbits during the 2-hour observation period. Yet the same injury produced less severe bleeding in normal animals, in each of which hemostasis developed and blood pressure stabilized during the second hour of the experiment. The final MAP in this group was 59.3 ± 20.3 mm Hg. The average blood loss and survival times of the rabbits are shown in Table 4. No difference in mortality rate or blood loss was found between the two coagulopathic groups. Kaplan-Meier analysis of survival time (Fig. 3) indicated significant difference between normal and coagulopathic rabbits ($p < 0.001$), but did not suggest any difference between the two coagulopathic conditions. The average survival times were 91 ± 9 minutes and 67 ± 5 minutes for W and HH rabbits, respectively, and 120 minutes for normal controls.

The coagulation data (bleeding time [BT], PT, aPTT, platelet, fibrinogen, and TEG measurements) were analyzed by multiple logistic regression analysis with stepwise selection procedure. Based on two prediction models with the area under receiver-operating curves equal to 0.938 and 0.979, the BT (odds ratio 1.03; 95% CI = 1.006–1.052) and Vmax (odds ratio 0.62; 95% CI= 0.363–1.044) were determined, respectively to be the best predictors of mortality in this study.

DISCUSSION

This study characterizes a simple and reproducible model of uncontrolled hemorrhage in a small animal. The model will be used for screening the safety and efficacy of a

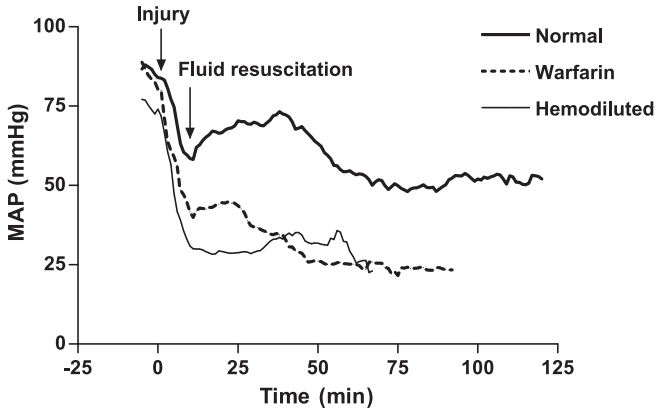

Fig. 2. *Averages of mean arterial pressure of normal and coagulopathic rabbit groups during the course of the experiments.*

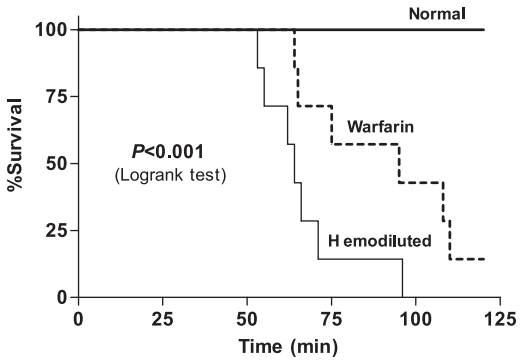

Fig. 3. *Kaplan-Meier analysis of survival time of different rabbit groups. There was no difference in survival time between warfarin and hemodiluted-hypothermic rabbits.*

variety of hemostatic agents and clotting proteins to enhance coagulation and correct coagulopathy. The use of new hemostatic agents administered alone or in combination with other therapies (e.g. resuscitation) might be associated with the risk of intravascular nonspecific thrombosis and its fatal consequences. Generally, high cost and significant manpower and resource requirements prohibit conducting these types of preliminary safety and efficacy studies in large animals particularly because some might turn out to be ineffective or unsafe. Therefore, a small animal model with trauma-relevant hemorrhage and associated complications (e.g. coagulopathy) might serve as a useful means for rapid and efficient screening of multiple agents. The promising agents will be further investigated in large animal studies.

In the past, several bleeding models have been developed in rats that were used for evaluating the effectiveness of topical hemostatic agents and different resuscitation regimens to control hemorrhage or improve survival, or both.[22–26] However, because of the limited blood volume of the rat, multiple blood sampling and serial measurements of coagulation parameters cannot be performed without adversely affecting the outcome of the experiments. We have selected the rabbit as an animal model for several reasons, including its large circulating blood volume (200–250 mL in 3–3.5 kg rabbits) that allows blood sampling (up to 10 mL) and coagulation measurements without significantly impacting the physiology of the animal. Other advantages are the docile nature of the rabbit and easily accessible ear arteries and veins for blood draws and IV drug injections. In addition, unlike rats, rabbits are very sensitive to thrombotic agents and are routinely used to evaluate the safety (thrombogenicity, immunogenicity, tissue toxicity) of various agents for use in patients.[27–29] Rabbits are also more sensitive to hemorrhage and respond better than rats do to most pro-coagulant agents.[30,31]

The splenic injury in this study produced significant bleeding and loss of approximately 30 mL/kg of circulating volume in normal rabbits. This degree of blood loss was tolerated and all the control rabbits survived given limited fluid resuscitation. The same injury in coagulopathic rabbits, however, caused a loss of nearly 53 mL/kg of circulating volume and 86% to 100% of the cases resulted in a fatality with the same resuscitation regimen. The coagulation derangement responsible for the large blood losses and fatality in coagulopathic animals was different in the two study groups. The warfarin treatment inhibited only the synthesis of vitamin K-dependent clotting factors, particularly factor VII with the shortest half life (4–6 hours), without affecting RBC, platelet, or fibrinogen concentrations. The TEG measurements of whole blood showed a significant delay in clot R-time, which coincided with prolonged PT, and decreased clot formation rate with no effect on the final clot strength. Therefore, it appears that the main reason for the lack of any sustainable hemostatic clot formation in this group was a result of the slow clotting process and possible washing away of any weak clot(s) that formed on the wound before it reached its maximum strength.

In the dilutional hypothermic coagulopathy group, which was produced by 50% hemodilution and 4°C temperature reduction, all the clotting proteins as well as RBC and platelets were diluted by at least 40%. TEG analysis revealed reduced clot formation rate, though not as much as in warfarin-treated animals, and a decrease in maximum clot strength. However, this type of coagulopathy had little or no effect on clot R-time or its equivalent PT as measured by the standard plasma coagulation tests. The changes were better reflected by the prolonged aPTT test, which measures intrinsic pathway clotting factors (e.g. factors XII, XI). Based on TEG analysis, it is apparent that the excessive bleeding in this coagulopathic model was caused by a slow clotting process and a decrease in maximum clot strength caused by the decrease in platelet count and fibrinogen concentration in the blood. Therefore, the slow forming and considerably weaker clot formed in this condition were unable to seal the vascular injury and stop the bleeding.

The reductions in the activity of factors II, IX, and X of the hemodiluted blood were equivalent to decreases seen in the warfarin-treated blood, except for factor VII that was less in the warfarin group (no difference vs. normal). This difference suggests that the marked decrease of factor VII activity (73%) was primarily responsible for the increase of PT in warfarin-treated animals, but the 45% reduction of factor VII activity was not sufficient to cause a significant delay in PT or its equivalent R-time in TEG assays in hemodiluted rabbits. The insensitivity of PT to demonstrate dilutional coagulopathy was found in other studies in which 60% of the circulating blood of pigs was exchanged and their body temperature was reduced by 5°C. In these cases, the PT was prolonged only by 1 to 1.5 seconds when it was measured at normal temperatures (37°–38°C).[32,33] The PT was prolonged by 6 seconds when the test was performed at 33°C (temperature effect only).[33] In another study, however, the PT remained unchanged even though it was measured at the hypothermic temperature of the pigs.[34] This difference might be a result of the use of different colloid solutions (Hextend vs. albumin) to produce hemodilution in those studies.

PT longer than 1.5 times normal is an indicator of coagulopathy in trauma patients who are at higher mortality risk.[6] However, the coagulopathy model that was produced by hemodilution and hypothermia in this study with normal PT is clearly different from those that are observed in some trauma patients with massive tissue injuries. In fact, Brohi et al. reported an acute traumatic coagulopathy in patients with an associated high mortality that was not related to fluid administration.[6] This coagulopathy reflected the severity of tissue damage and was diagnosed by early coagulation screening tests (prolonged PT, aPTT, and TT) before significant fluid was administered. The hemodilution hypothermia derangement produced in the rabbits or other experimental animals also leads to coagulopathic bleeding and high

mortality,[32–34] but it lacks the initial massive tissue injuries or acidosis that are seen in some trauma patients. This hypothermia and hemodilution type of coagulopathy is better diagnosed by the methods that measure the kinetics of clot formation and the final strength of the blood clot, rather than the initial reaction time as measured by PT or aPTT (the standard clinical diagnosis of coagulopathy). The results of this study might also suggest a possible subgroup of trauma or surgical patients with excessive fluid transfusion who are at the risk of excessive bleeding but misdiagnosed for normal coagulation function because of their normal or near normal PT values. The variability of PT and aPTT tests to diagnose increased surgical bleeding and guide treatment of dilutional coagulopathy was reported in a prospective study of patients undergoing extensive spinal surgery.[35] A recent review of the literature by Segal et al. also concluded that there is insufficient evidence to conclude that the prolonged PT or elevated INR can predict excessive bleeding in patients undergoing invasive procedures.[36] Although abnormal PT and aPTT test results in trauma patients are shown to be highly correlated with increased mortality rate, the causes of death in these patients were not identified as excessive bleeding.[6–8]

Logistic regression analysis of all the coagulation data collected from all the rabbits (normal and two coagulopathic groups) showed that the more sensitive predictors of lethal hemorrhage among all three groups were the in vivo BT and the in vitro maximum clotting velocity (Vmax) measured by TEG. Although BT is a useful tool for investigating the hemostasis process, as seen in the present study, its utility as a predictor of the high risk of hemorrhage in a clinical setting remains doubtful.[37,38] There are widely disparate mechanisms that can produce an increase in the BT and the test result should be interpreted differently depending on each instance. For example, despite equal BTs, the risk of blood loss in a trauma patient (otherwise healthy) might be different from in the patients with renal failure, liver failure, or thrombocytopenic purpura. Moreover, there is no evidence that the bleeding from a standardized cut in the skin (clinical BT test) reflects the risk of bleeding elsewhere in the body.[38] Alternatively, TEG analysis of whole blood provides accurate and detailed information about the hemostatic capacity of the patient's blood, which might be superior to both BT and standard coagulation tests even though it might take more time to perform. TEG results might also serve as a better predictor of excessive bleeding and high risk of mortality in trauma patients.

In summary, we have developed an uncontrolled hemorrhage model involving a simple and reproducible splenic injury in rabbits. The coagulation components, screening tests, and bleeding outcomes were measured in the model using both normal and coagulopathic animals. The coagulopathy was produced by two different methods; pretreatment with warfarin or hemodilution and hypothermia induction before injury. The splenic injury caused a 32% blood loss and zero mortality in normal rabbits, but caused 55% blood loss and 84% to 100% mortality in coagulopathic rabbits. The standard PT test, performed before injury, was sensitive to warfarin treatment and increased 1.9-fold but it was unresponsive to the dilutional and hypothermia coagulopathy. Bleeding time and clotting rate measurement by TEG were better indicators of coagulopathic bleeding and mortality in both coagulopathic models.

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DISCUSSION

Dr. Lewis M. Flint (Tampa, Florida): As I understand, the purpose of the study was to attempt to develop a screening laboratory test that would predict coagulopathy and death in a laboratory animal preparation, which has been designed to mimic the coagulopathy associated with injury, shock, acidosis, and hypothermia.

With regard to the objective of the article, it is clear that the development and testing of agents designed to improve trauma-related coagulopathy will require dependable animal models. The task before us is to determine if this model is adequate as it stands; a step in the right direction; or a stimulus to go back to the drawing board. For me, the answer to these questions must begin with a determination of the faithfulness of the model to the disease state being studied. I was first introduced to trauma-related coagulopathy on a large scale on days 2 and 3 of the 1968 Tet offensive. On those days, the surgeons at the 71st Evacuation Hospital in Pleiku South Viet Nam processed 327 casualties in a single 24-hour period and performed 218 operations under general anesthesia. On two occasions we were temporarily without blood for transfusion and, of necessity, subjected patients to hemodilution and the hypothermia that accompanied that means of resuscitation since we did not have fluid warmers. We ran out of lactated Ringer's solution on one occasion and resuscitated patients with normal saline which regularly produced a hyperchloremic acidosis. What amazed me was my observation that many patients developed acidosis and hypothermia but few patients developed abnormal bleeding so long as we were able to stop the bleeding in the wound sites, debride dead tissue, and reverse shock-related perfusion deficits. Subsequently, I have observed many trauma patients who have prolonged prothrombin times and low platelet counts after definitive operative treatment of their injuries or after "damage control" procedures who are not showing abnormal bleeding. I have not diagnosed coagulopathy unless the patient is bleeding from sites other than the operative site or wound sites.

My first question is, is the coagulopathy associated with trauma a disease, a syndrome, or merely a symptom of inadequate hemostasis and/or incompletely resuscitated shock? My second question is that since your animals did not bleed from any site other than the wound you produced, have you faithfully reproduced the clinical features of the disease, syndrome, or symptom?

There is no doubt that your experiments produced a multifactorial process resulting in abnormal coagulation studies. The abnormal tissue bleeding time is a suggestion of thrombocytopenia or platelet dysfunction but your data do not regularly show platelet counts below 100,000, which we associate with clinical coagulopathy. Do you have any data on platelet function? The abnormal thromboelastograms re-

flect a global reduction in the efficiency of the clotting process. There was no data offered on evidence of increased fibrinolysis or histologic evidence of diffuse intravascular coagulation. Do you have any data on these aspects of coagulopathy?

Finally, what happens if you stop the bleeding before death of the animal stops it? Would the animal then show abnormal clotting tests and, possibly bleeding from other sites or abnormal clotting studies with no bleeding? The bottom line for me is, if there is no unexpected bleeding, do you really have trauma-related coagulopathy? You have chosen to study a tough problem.

Dr. Bijan S. Kheirabadi (Ft. Sam Houston, Texas): With respect to what percent of the casualties have an increased PT time and develop coagulopathy, the next presentation will address that.

With respect to the question of if the coagulopathy is a disease, a syndrome, or merely a symptom of hemorrhage and incomplete resuscitation, my belief is that coagulopathy is induced by massive tissue injury, large hemorrhage that dilutes and consumes most coagulation factors and hypoperfusion, which causes metabolic acidosis and hypothermia. The hemodynamic changes require fluid resuscitation, which further dilute the coagulation factors.

Did our model faithfully reproduce all the aspects of the trauma-related coagulopathy? No, and I have addressed that in our article. If there was a limitation, it was that our model did not have that initial massive tissue injury. However, we have subjected our animals to a controlled hemorrhage, full resuscitation, hypothermia, and a mild acidosis. Therefore, we have incorporated most components of coagulopathy into the model. These changes were severe enough to cause coagulopathic bleeding that led to exsanguination of all tested animals. All the models have some limitations. This model would be appropriate when an initial tissue injury is incorporated into procedures.

Whether the animals were just bleeding from splenic injury or there was, indeed, coagulopathy present in the animals, these animals were indeed coagulopathic because during subsequent laparotomy, it was much harder to control diffuse bleeding from the abdominal muscle incision than in the normal animals and required more cautery to stop the diffuse bleedings.

In terms of the bleeding time being prolonged and yet platelet counts were normal in warfarin-treated rabbits, this was exactly our finding. We think that increased bleeding time does not reflect just the decrease of platelet counts. In this case, the slow clot formation, as seen by TEG analysis, would also lead to a longer bleeding time in in-vivo measurement.

We did look at the fibrinolysis, but we did not see any difference between normal and hemodiluted hypothermic rabbits. The fibrinolysis appeared normal in both groups.

With respect to the question of did we stop the bleeding and observe the animals being actually coagulopathic, we did not. However, in the animals that survived beyond one hour, we took a blood sample after one hour of bleeding and resuscitation, and we measured the coagulation in these cases. Both PT and aPTT indicated coagulopathy and progression of the coagulopathy was clear in that situation.

Dr. Frederick A. Moore (Houston, Texas): When we created our massive transfusion at Memorial Hermann Hospital in Houston, we reviewed the literature and it was apparent that we really don't understand posttraumatic coagulopathy. I was taught that posttraumatic coagulopathy occurs as we resuscitate the severely injured bleeding patient and that the contributing factors are hemodilution, consumption of clotting factors, acidosis, and hypothermia. However, several recent studies have documented significant coagulopathy (elevated PT) in severe trauma patients upon arrival to the trauma center before these events have occurred. Severe trauma is activating the coagulation cascade.

Does your model really reflect what we're seeing clinically? Do you need to modify your model? How about adding a soft tissue injury or liver injury?

My next comment concerns the clinical challenge of identifying that coagulopathy very early after arrival in the emergency department (ED). Is TEG the right tool to be using in the ED? Could you comment on the variability of TEG measurements across institutions?

Dr. Bijan S. Kheirabadi: With respect to injury, that is exactly what we plan to do. By introducing an early tissue injury, perhaps some kind of a liver injury, bring the massive tissue injury to this process and incorporate that aspect also in our coagulopathy model.

With regard to TEG, I think that TEG testing was certainly the best predictor of these coagulopathic situations. It takes about 20 minutes to do the test. However, if you think about the speed of aPTT or PT tests, each test also takes at least 10 minute for blood centrifugation and subsequent assays. So TEG takes slightly longer. But there are ways to speed up the test by adding more tissue factor and other stimulants. Among all the assays that we have run, we found TEG to be the best predictor of coagulopathic condition before we actually observe the hemorrhage and mortalities.

I believe one possibility for variability of TEG results is the use of citrated blood samples and variability in the incubation time that both affect the TEG measurements. If fresh blood can be tested at the time that it has been drawn (no anticoagulant), it will give a more consistent results.

Dr. Karim Brohi (London, England): I applaud you for promoting the use of TEG, which undoubtedly picks up more about the clotting system than we are getting from PT and PTT.

But I, too, would question your model in terms of its applicability to trauma patients. The articles that you quoted both identified an early coagulopathy that was unrelated to dilution.

Both of them, however, showed a coagulopathy with minimal prehospital fluid resuscitation. It is clear to us that you need a tissue injury as well as shock to produce coagulopathy. This is probably related to an interaction between the

extrinsic pathway and activation of anticoagulant pathways such as the protein C pathway, and we expect to publish this fairly shortly.

But I think that, without that tissue injury, your results are true for dilution, but not true for trauma. And I think the subsequent time point measures that you just talked about are far more indicative of what we are actually seeing than the data that you have presented today.

Dr. Bijan S. Kheirabadi: Yes, I totally agree with you. I have tried to divide the coagulopathy into several components. One component is definitively involved with tissue injury that probably initiates the whole coagulopathy process. But the other components that come after that, such as hemorrhage and resuscitation, certainly exaggerate coagulopathy and make the situation harder to control and cause more bleeding and death.